Lamellar Body Count and Fetal Respiratory Distress Syndrome

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Conflict of Interests

There are no conflicts of interest to declare.
Resources and Citations

There are several great resources for information on Fetal Lung Maturity Testing

- David Grenache, PhD – ARUP – Salt Lake City
- FLMoptions.com
- Anne Gronowski, PhD – Washington University School of Medicine – St. Louis
- AACC Pediatric and Maternal-Fetal Division

All of these sources were used in the preparation of this program. Their contributions are gratefully acknowledged.

Fetal Respiratory Distress Syndrome

- AKA – Hyaline Membrane Disease
- Clinical Manifestations of FRDS
  - Tachypnea – rapid shallow breathing
  - Prominent, audible grunting
  - Nasal Flaring
  - Sub-costal and inter-costal retractions
  - Cyanosis unresponsive to O2 administration
  - Progressive worsening of cyanosis and dyspnea.
  - Apnea and irregular respirations
FRDS Risk Factors

- Prematurity (<37 weeks)
- Male gender
- Caucasian race
- 2nd born infant of twins
  - More frequent in multiple births
  - History of FRDS in sibling

RDS Causes and Surfactant Composition

- Caused by a deficiency in pulmonary surfactant
- Pulmonary surfactants decrease surface tension of water
- Composition of surfactants
  - 85% phospholipids
    - Phosphatidylcholine - 76%
    - Phosphatidylglycerol - 13%
    - Phosphatidylinositol - 4%
    - Phosphatidylethanolamine - 3%
    - Sphingomyelin 2% and other phospholipids 2%
  - 10% protein
  - 5% neutral lipids
FRDS Incidence

- Most common cause of respiratory failure in neonates
- Incidence is indirectly proportional to gestational age at delivery
  - 60-80% of births at <28 weeks have FRDS
  - 15-30% of births at 32-36 weeks have FRDS
  - 5-6% of births at 37 weeks have FRDS
- FRDS of Full Term deliveries is rare

Ref: Grenache, David

Tests for FLM

For detail, refer to “Contemporary Issues in FLM Testing” presentation by David Grenache, PhD.

- Criteria for FLM Test Selection:
  - Rapid performance
  - Must have high sensitivity for immaturity and high predictive value for maturity
  - Used for clinical decision making
  - Performed on Amniotic Fluid at 32 to 38 weeks of gestation (ACOG – 2008)
Tests for FLM

- **Precursor Tests**
  - L/S Ratio was the “gold standard” but of questionable reproducibility and accuracy. Was developed in the early 1970’s.
  - PG by TLC was slow and tedious.
  - PG by agglutination technically easy, but not very sensitive – developed early 1980’s
  - S/A ratio – (Abbott FLM) – automated test developed in the late 1980’s and will no longer be available. Sensitive, quantitative
  - LBC – first described in late 1980’s but not commonly performed. Sensitive, quantitative

Lamellar Body Count

- Lamellar bodies are similar in size to blood platelets (1-5 uM or 2 -10 fL).
- They can be accurately quantified using routine hematology analyzers.
- They are formed by the Type II pneumocytes through a “packaging” process and stored as granular material.
- They are excreted from the fetal lung into the amniotic fluid.
Lamellar Bodies

Human Platelets
Lamellar Body Counts

- **Advantages**
  - High Sensitivity for immaturity
  - Quick and simple to perform
  - Require low sample volume
  - Instruments are readily available in labs

- **Disadvantages**
  - Affected by blood and meconium
  - Lab developed test - not FDA approved
  - Instrument specific cutoffs required
    - Varies by model within manufacturers

Lab Consensus Guidelines
(Neerhoff, et. al.)

- Amniotic Fluid should NOT be centrifuged
- Grossly bloody or contaminated Amniotic Fluid should NOT be used
- **Cutoffs are Instrument and Model dependent** regardless of manufacturer
  - For most analyzers, range is:
    - Mature = >50,000/uL
    - Intermediate/transitional = 15,000 to 50,000/uL
    - Immature = <15,000/uL
Consensus Range Cautions

- Consensus range was established without regard of the hematology analyzer used.
- Not all analyzers use the same parameters for counting platelets; thus, it is **IMPERATIVE** that analyzer specific cut-off values be determined by the laboratory.

Pre-Analytic Considerations

- Amniotic Fluid (AF) is a heterogeneous mixture containing sloughed cells, hair and other fetal debris.
- It should be tested promptly (< 4 hours) after collection for best results.
- It should not be centrifuged prior to analysis. It should be gently mixed well, not vortexed.
- Grossly bloody or contaminated AF should be rejected.
Comparison of Various Analyzers

- Most early studies were performed using different models of Beckman Coulter analyzers.
  - These analyzers use electrical impedance differences through a 50 μM aperture.
  - Multiple studies have shown this technology to compare favorably with previous FLM tests (L/S ratio, PG, FLM/SA).
  - LBC is faster and easier to perform than other quantitative methods.

Comparison of Various Analyzers

- When compared to the Beckman Coulter Gen S counter, various studies found:
  - Concordance Rates as follow:
    - Sysmex XE-2100 = 86%
    - Siemens Advia 120 = 78%
    - Abbott Cell-Dyn 3500 = 66%

- Manufacturers use different apertures and various impedance mechanisms.

Ref: Lu, Gronowski, et.al.
Various Analyzer Maturity Cut-Offs
(suggested from literature)

- Beckman Coulter (GenS, STKR)
  - 50,000/µL
- Sysmex (XE-2100, K-800)
  - 50,000/µL except XE-5000
- Siemens (Advia 120)
  - 50,000/µL
- Abbott (Cell Dyn 3500)
  - 80,000/µL

Ref: Lu, Gronowski, et.al.

Analyzer Differences

- Sysmex
  - Uses electrical and radio frequency impedance plus an 80 uM aperture in addition to forward and side scatter and hydrodynamic focusing.
  - For all except XE-5000, they compare very well with the consensus ranges.

- Siemens
  - Uses low angle (volume) and high angle (refractive index) light scatter from a laser. Any particle with a volume of <60 fL and a high refractive index. They compare well with the consensus ranges.

- Abbott
  - Combine optical scatter and electrical impedance. They have a positive slope compared to the other analyzers and thus use a higher cutoff value for maturity than the consensus ranges.
Sysmex XE-5000

- Uses different software parameters for the platelet count.
  - Where most analyzers size the platelet parameter down to 1 fL or less, the XE-5000 sizes platelets using a 2 fL low discriminator.
  - Thus, LBCs on the XE-5000 will tend to be as much as 50% lower in some cases.
- This led to our study comparing LBC and L/S to FRDS at delivery.
- NOT FDA Approved – Lab Developed Test

McKennon XE-5000 FRDS study

- Study performed on 68 patients
- Compared LBC, L/S and RDS at delivery
- There was 1 still born, 1 twin delivery and 2 cases with 2 specimens submitted.
- Summary of specimen results:

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<th>LBC</th>
<th>L/S</th>
<th>RDS</th>
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<tr>
<td>Mature</td>
<td>52</td>
<td>25</td>
<td>56 none</td>
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<tr>
<td>Intermed</td>
<td>4</td>
<td>18 (m/caut)</td>
<td>-</td>
</tr>
<tr>
<td>Immature</td>
<td>13</td>
<td>5</td>
<td>12 RDS</td>
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McKennan Comments

- Both of the twins had respiratory distress
- All of the newborns with RD admitted to NICU
- The still born fetus had meconium contaminated AF so could not be tested.
- Several immature by LBC were called “mature w/caution” by L/S ratio.

McKennan Cut-offs for XE-5000

- Cut-offs validated for XE-5000 at AMcK.
  - Mature = >25,000/uL
  - Indeterminate/transitional = 21 to 24,000/uL
  - Immature = < 21,000/uL

Validated for our analyzer(s) in our lab
Proficiency Survey

- CAP Survey  LBC-A (May 2011)
  - Ungraded Educational Challenge
  - Demonstrated that different analyzers give different results on the same sample
  - Lumps instrument by manufacturer without consideration of model. (Issue for Sysmex)
    - XE-5000 lumped in w/ other Sysmex analyzers
    - Affects the Mean, SD and CV

LBC-A

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Summary

- FLM II assay by Abbott will no longer be available.
- L/S ratio is labor intensive, uses very toxic reagents and requires expertise. It is not standardized.
- Fetal Lung Maturity Testing is not indicated in the majority of pregnancies—only in premature deliveries <38 weeks with a few exceptions.

Summary

- LBC is an excellent predictor of fetal lung maturity and FRDS.
- Can be performed on most hematology analyzers.
- Test is NOT FDA approved – must be validated in your lab.
- Cut-off values vary by manufacturer as well as by instrument model.
Questions??

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